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TEXT SEARCHABLE DOCUMENT - 2010

Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-6 to Algae (*Selenastrum capricornutum*)

PMRA Submission Number {.....} EPA MRID Number 47807006

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	367525
	OECD Data Point	{.....}
	EPA MRID	47807006
	EPA Guideline	OPPTS 850.5400 (123-2)

Test material: **Trifluralin Metabolite TR-6** **Purity:** 99%
Common name
Chemical name: IUPAC 3,4-diamino-5-nitro-triflluoromethylbenzene
CAS name 1,2-Benzenediamine, 3-nitro-5-(trifluoromethyl)
CAS No. Not reported
Synonyms Not reported

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental **Date:** 11/2/09

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental **Date:** 12/02/09

Primary Reviewer: Christine Hartless **Date:** 4/21/10
EPA/OPP/EFED/ERB 1 4-21-10

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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Use Site Category:	{.....}	[For PMRA]
EPA PC Code	036101	

Date Evaluation Completed: 4/21/10

CITATION: Henry, K.S., Staley, J.L., and E.L. McClymont. 2002. Trifluralin metabolite TR-6: Growth inhibition test with the freshwater green alga, *Selenastrum capricornutum* PRINTZ. Unpublished study performed by The Dow Chemical Company, Toxicology and Environmental Research and Consulting, Midland, Michigan. Laboratory Study ID: 011101. Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed January 15, 2002.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater green algae (*Selenastrum capricornutum*) were exposed to Trifluralin metabolite TR-15 at nominal concentrations of 0 (negative and solvent control), 0.078, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10 mg ai/L under static conditions. The arithmetic mean-measured concentrations were <0.01 (<LLQ, negative and solvent control), 0.065, 0.156, 0.300, 0.613, 1.17, 2.40, 4.79, and 5.56 mg ai/L.

Cell abnormalities were not reported.

This study is scientifically sound and is classified as Supplemental for the freshwater green algae, *Selenastrum capricornutum* and the trifluralin degradate TR-6. Reported results can be used qualitatively in risk characterization but cannot be used quantitatively for risk estimation.

All endpoints were affected by the test material. Biomass was the most sensitive endpoint, with NOAEC and EC_{50} values of <0.065 and 4.6 mg ai/L, respectively. The reviewer's analysis detected a significant effect ($p<0.05$) of the solvent on algal cell density and biomass parameters and noted that the fit of the Bruce-Versteeg model to the data for cell density and biomass was poor and not representative of the raw data.

Test Organism: *Selenastrum capricornutum*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
IC_{05}: NA* 95% C.I.: NA
IC_{50}: 5.4 mg ai/L 95% C.I.: 4.8 to 6.1 mg ai/L
Slope: 3.09 ± 0.653
NOAEC: 0.156 mg ai/L
*NA – value estimated by the Bruce-Versteeg method was not representative of the data and the reviewer recommends not reporting or using this value

Biomass (Area Under the Growth Curve)
IC_{05}: NA* 95% C.I.: NA
IC_{50}: 4.6 mg ai/L 95% C.I.: 4.1 to 5.1 mg ai/L
Slope: 3.39 ± 0.624
NOAEC: <0.065 mg ai/L
*NA – value estimated by the Bruce-Versteeg method was not representative of the data and the reviewer recommends not reporting or using this value

Growth Rate
IC_{05}: 4.5 mg ai/L 95% C.I.: 3.8 to 5.3 mg ai/L
IC_{50}: >5.56 mg ai/L 95% C.I.: N/A
Slope: 5.69 ± 2.32
NOAEC: 0.156 mg ai/L

Endpoint(s) Effected: Cell density, biomass, and growth rate

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following the OECD Guideline for Testing of Chemicals, No. 201: "Algal, Growth Inhibition Test", the EEC Commission Directive 92/69/EEC Annex, C.3 Algal Inhibition Test, U.S. EPA Pesticide Assessment Guidelines Subdivision J Hazard Evaluation: Non-target Plants Guideline 123-2, and EPA Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2. The following deviations from OPPTS 850.5400 were noted:

1. There were significant reductions ($p<0.05$) in cell density (5%) and biomass (15%) in the solvent control, relative to the negative control. Growth rate controls did not significantly differ from one another. EPA guidance suggests that study acceptability may be impacted if the solvent is shown to have an effect on organism response. Given the endpoints affected and the magnitude of the difference (5 to 15%), the reviewer is uncertain if the response in the treated groups was a function of the test material alone.
2. The pretest health of the algae was not reported; the reporting of this parameter is suggested by OPPTS guidelines.
3. The source of the dilution water was not reported.
4. Water characterization analysis was not performed or reported, resulting in a lack of data for total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water; the reporting of these dilution water parameters is suggested by OPPTS guidelines.
5. The lighting quality was not reported.
6. The lighting intensity in the definitive test was much higher than recommended, and ranged from 6280 to 7980 lux; OPPTS guidelines suggest that the intensity for *Selenastrum capricornutum* be maintained at 4300 lux.
7. The pH in the definitive test was much higher than recommended by OPPTS guidelines, and ranged from 7.5 to 9.0; OPPTS guidelines suggest a pH of 7.5 ± 0.1 for tests with *Selenastrum capricornutum*. The study authors did measure pH in test solutions without algae present, and obtained a range of 7.5 to 8.4.

These deviations do impact the classification of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160), OECD Principles of GLP (ENV/MC/CHEM(98)17; 1997), and EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

A. MATERIALS:

1. Test material Trifluralin Metabolite TR-6

Description: Solid

Lot No./Batch No. : GHD-6140-36A

Purity: 99%

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Stability of compound under test conditions: The time 0 measured concentrations yielded recoveries ranging from 53 to 113% of the nominal test concentrations, and the 96-hour measured concentrations yielded recoveries ranging from 58 to 99% of nominal and 77 to 109% of initial. Trifluralin Metabolite TR-6 recovery was consistant in all test concentrations. However, at the highest nominal concentration (10 mg ai/L), recovery was 53 and 58% at 0-hr and 96-hr time points, respectively. It may be inferred that the solubility limit had been reached at this highest concentration.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of Trifluralin Metabolite TR-6.

Parameter	Values	Comments
Water solubility at 20°C	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Name: Freshwater green algae, *Selenastrum capricornutum* Printz

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 1648
Source: In-house cultures originally obtained from the University of Toronto Culture Collection, Toronto, Ontario, Canada
Age of inoculum: 4 weeks acclimation (3 days since previous transfer to fresh medium)
Method of cultivation: Cultivated under test conditions (algal assay medium; AAM)

B. STUDY DESIGN:

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1. Experimental Conditions

a. Range-finding study A range-finding study was conducted for 3 days with test concentrations of 0.016, 0.08, 0.4, 2, and 10 mg ai/L. The NOAEC was between 0.08 and 0.4 mg ai/L, and the EC_{50} value was between 0.4 and 2 mg ai/L.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	
Culturing media and conditions: (same as test or not)	Algal assay medium Temperature, photoperiod, medium were the same; light intensity was different (4300 for culturing, 8000 for testing)	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Health: (any mortality observed)	Pretest health was not reported.	
Test system Static/static renewal	Static	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Renewal rate for static renewal	N/A	
Incubation facility	Test vessels were placed in an incubator/environmental growth chamber.	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)*	Glass	

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Parameter	Details	Remarks
		Criteria
Size: Fill volume:	250 100	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	AAM Not reported Not reported Only for culturing $NaHCO_3$ N/A	pH range throughout entire study: 7.5-9.0 *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	A standard nutrient medium was prepared, and a detailed composition was provided.	

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Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water Adjusted to 7.0-7.5 N/A Not reported Not reported Not reported Not reported Not reported Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test substance (200 mg) was dissolved in acetone (2 mL). The stock solution was serially diluted to prepare the remaining dose stock solutions. Aliquots (50 µL) of each of the dose stock solutions were added to the algal assay medium (500 mL) to prepare bulk dose solutions (final test concentrations).	
Aeration or agitation	Agitation (100 rpm)	
Initial cells density	*Ca.* $0.8x10^4$ cells/mL (average = $0.79x10^4$ cells/mL)	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

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Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	 3 3 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal: Mean-Measured:	 0 (negative and solvent control), 0.0780, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10 mg ai/L <0.01 (<LLQ, negative and solvent control), 0.065, 0.156, 0.300, 0.613, 1.17, 2.40, 4.79, and 5.56 mg ai/L	LLQ = lowest level quantified *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Acetone, < 0.1%	
Method and interval of analytical verification	Samples from test solutions collected at 0 and 96 hours, method precision samples from day 0, and concurrently run analytical standards were analyzed using HPLC with UV (285 nm) detection.	

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Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	24-24.5°C Continuous 6280-7980 lux Lighting not described	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- (algal growth) cell density -% inhibition of growth (biomass) -growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

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Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell counts were conducted daily on samples of each test concentration and the controls using an electronic particle counter (Coulter Multisizer flow cytometer). Biomass and growth rate calculations were not reported.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes. After 96 hours, the mean cell density was 361 x 10^4cells/mL in the negative control.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Only for cell density.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, cell density averaged 361 and 343 x 10^4 cells/mL in the negative and solvent control, respectively, yielding inhibitions of 0, 0, 15, 5, 13, 16, 43, and 56% as compared to the pooled controls in the mean-measured 0.065, 0.156, 0.300, 0.613, 1.17, 2.40, 4.79, and 5.56 mg ai/L treatment levels, respectively. The 96-hr NOAEC and EC_{50} values for cell density were 1.17 and 5.53 mg ai/L, respectively.

The study authors did not report averages, replicate data, or inhibitions for biomass and growth rate. The reviewer used cell density data to calculate biomass and growth rate per replicate, and then used mean values per replicate to calculate inhibitions relative to the negative control.

After 96 hours of exposure, area under the curve (biomass) averaged 1017 and 863 x 10^5 cells/mL in the negative and solvent control, respectively, yielding inhibitions of 17, 20, 22, 16, 20, 30, 58, and 69% as compared to the negative control. The 96-hr NOAEC and EC_{50} values for biomass were 1.17 and 10.9 mg ai/L, respectively.

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After 96 hours of exposure, growth rate averaged 0.065 and 0.061 cells/mL in the negative and solvent control, respectively, yielding inhibitions of 0, 3, 5, 5, 5, 2, 9, and 15% as compared to the negative control. The 96-hr NOAEC and EC_{50} values for growth rate were 1.17 and >5.56 mg ai/L, respectively.

Cell abnormalities were not reported. The study authors determined toxicity values using mean-measured concentrations.

Table 3: Effect of Trifluralin metabolite TR-6 on algal growth (*Selenastrum capricornutum*)

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial cell Density ($x10^4$ cells/mL)	Cell density ($x10^4$ cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition*
Negative control	0.7	7.1	37.5	201.3	361.0	NA
Solvent control	1.0	5.6	26.2	160.1	342.7	5
0.065 (0.078)	0.7	5.9	21.1	151.6	351.3	3
0.156 (0.156)	0.8	5.0	20.3	141.7	352.5	2
0.300 (0.313)	0.8	5.0	27.3	151.1	300.6	17
0.613 (0.625)	0.9	6.1	30.8	157.6	333.1	8
1.17 (1.25	0.8	5.4	28.6	152.6	306.4	15
2.40 (2.50)	0.7	4.9	22.5	126.1	294.4	19
4.79 (5.00)	0.7	3.6	13.3	63.3	200.9	44
5.56 (10)	0.8	2.7	10.3	44.4	155.8	57

*Inhibitions calculated by the reviewer; determined by comparison to the negative control.

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Table 4: Effect of Trifluralin metabolite TR-6 on algal growth (*Selenastrum capricornutum*)

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (cells/mL)		Mean Area Under the Growth Curve (Biomass) ($x\ 10^5$ cells/mL)	
		0-96 Hours	Percent Inhibition*	0-96 hours	Percent Inhibition*
Negative control	0.7	0.065	N/A	1017	N/A
Solvent control	1.0	0.061	6	863	15
0.065 (0.078)	0.7	0.065	0	844	17
0.156 (0.156)	0.8	0.063	3	817	20
0.300 (0.313)	0.8	0.062	5	794	22
0.613 (0.625)	0.9	0.062	5	859	16
1.17 (1.25	0.8	0.062	5	809	20
2.40 (2.50)	0.7	0.064	2	716	30
4.79 (5.00)	0.7	0.059	9	427	58
5.56 (10)	0.8	0.055	15	318	69

*Inhibitions calculated by the reviewer; determined by comparison to the negative control.

Table 5: Statistical endpoint values – 96 hours.

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC_{05} (mg ai/L)	1.17	1.17	1.17
EC_{50} (mg ai/L)	5.53	10.9	>5.56
IC_{50} or EC_{50} (mg ai/L) (95% C.I.)	5.53 (3.77-7.28)	10.9 (0.23->100)	>5.56 (N/A)
Other (EC_{25})	2.81 (1.15-4.47)	ND	ND
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

ND – not determined

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Table 6: Statistical endpoint values – 72 hours.

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC_{05} (mg ai/L)	1.17	1.17	1.17
EC_{50} (mg ai/L)	4.09	8.19	>5.56
IC_{50} or EC_{50} (mg ai/L) (95% C.I.)	4.09 (2.25-5.92)	8.19 (0.06->100)	>5.56 (N/A)
Other (EC_{25})	2.06 (0.27-3.85)	ND	ND
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

ND – not determined

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints cell density, biomass, and growth rate using the pooled negative and solvent controls and the mean-measured concentrations. The EC_{50} for biomass was calculated by regression of the differences in area under the growth curves for each dose group compared to the control group against the log of the concentrations for days 3 and 4. The EC_{50} for growth rate was calculated by regressing the percent reduction in average growth rate for each dose group compared to the control group against the natural logarithm of the concentrations for the 0 to 72-hour and 0 to 96-hour exposure periods. The EC_{25} and EC_{50} values for cell density were determined by a least squares linear regression of algal cell counts against the log of the concentration on days 3 and 4. The NOAECs for algal growth were determined using ANOVA and Dunnett's test ($\alpha = 0.05$).

C. VERIFICATION OF STATISTICAL RESULTS: Statistical Method: Replicate data for all endpoints were assessed to determine toxicity values. Cell density and biomass values were entered into Toxstat as the value x 10^4 and x 10^5, respectively. Growth rate data were multiplied by 1,000 before entry into Toxstat. The negative and solvent controls were compared using a t-test before further analysis. For cell density and biomass data, the t-tests detected a statistically significant difference ($p<0.05$) between the control groups, with the solvent control having a reduction in the response variables relative to the negative control. There were no statistically significant differences between the two control groups for growth rate.

Using Toxstat 3.0, the reviewer tested the cell density, biomass, and growth rate replicate data for normality using Shapiro Wilk's test and for homogeneity of variance using Hartley and Bartlett's test. The data met the assumptions of ANOVA, therefore the NOAEC was determined using Dunnett's and William's tests.

The ICx values (with 95% C.I.) and probit slope was determined using probit analysis via Nuthatch Statistical Software.

All toxicity values were determined using the 96-hour mean-measured concentrations.

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Based on the IC_{50}, biomass was the most sensitive parameter. However, the NOAEC was undefined at < 0.065 mg/L (the lowest test concentration). Typically, the IC_{05} would be estimated from Nuthatch (Bruce-Versteeg methodology); however, in this case, the fit of the model to the data was very poor and not representative (see figure at end of appendix. The model predicts the IC_{05} to be at 1.5 mg/L; however, raw data from all test concentrations indicate at least a 10% reduction from the control. The reviewer has little confidence in the IC_{05} estimate for biomass because of poor model fit to the data and because of a significant solvent effect. The reviewer has the same concerns regarding the IC_{05} estimate for cell density and recommends it not be reported as well.

Cell density (reviewer has no confidence in the IC_{05} estimate)
IC_{05}: 1.6 mg ai/L 95% C.I.: 0.95 to 2.7 mg ai/L
IC_{50}: 5.4 mg ai/L 95% C.I.: 4.8 to 6.1 mg ai/L
Slope: 3.09 ± 0.653
NOAEC: 0.156 mg ai/L

Biomass (Area Under the Growth Curve, reviewer has no confidence in the IC_{05} estimate)
IC_{05}: 1.5 mg ai/L 95% C.I.: 0.93 to 2.4 mg ai/L
IC_{50}: 4.6 mg ai/L 95% C.I.: 4.1 to 5.1 mg ai/L
Slope: 3.39 ± 0.624
NOAEC: <0.065 mg ai/L

Growth Rate
IC_{05}: 4.5 mg ai/L 95% C.I.: 3.8 to 5.3 mg ai/L
IC_{50}: >5.56 mg ai/L 95% C.I.: N/A
Slope: 5.69 ± 2.32
NOAEC: 0.156 mg ai/L

D. STUDY DEFICIENCIES:

There were significant differences between the negative and solvent controls for cell density and biomass data ($p<0.05$; 5 and 15%, respectively). The US EPA guidance memo entitled, "Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies" (September 25, 2008) states that the magnitude of the difference between the controls and the prevalence of the response across multiple endpoints could justify a reduced classification of the study, if there is evidence to suggest interference of the solvent with organism response to the test material. In this study, the two most sensitive endpoints were impacted, with the most sensitive (biomass) being reduced 15% in the solvent control. Given the fact that cell density was also significantly impacted (5% reduction in the solvent control, compared to the negative control) and consideration of the magnitude of the reductions in the treated groups, the reviewer cannot be certain that effects on these endpoints were due to the test material alone.

E. REVIEWER'S COMMENTS:

The reviewer's results were different from the study authors', with the exception of the EC_{50} value for cell density. Both the reviewer and the study authors used mean-measured concentrations to calculate toxicity values. However, the study authors did not report how they calculated biomass and growth rate, nor did they provide mean values for any time interval. Further, the reviewer's results indicate that biomass is the most sensitive endpoint, while the study authors' results indicate that cell density is the more sensitive. Therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

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The test material was soluble in all concentrations except the highest test level, where nominal concentrations were less than 60% at time 0. However, TR-6 did not degrade at this highest level, resulting in similar recoveries at test termination. The reviewer chose mean-measured concentrations for determination of toxicity values and reporting in this DER.

The study authors did not provide biomass or growth rate replicate or mean value data, or % inhibitions. The reviewer independently calculated the replicate data for these endpoints using cell density data in Excel 2003.

The pretest health of the algae was not reported; the reporting of this parameter is suggested by OPPTS guidelines.

The source of the dilution water was not reported.

Water characterization analysis was not performed or reported, resulting in a lack of data for total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water; the reporting of these dilution water parameters is suggested by OPPTS guidelines.

The lighting quality was not reported.

The lighting intensity in the definitive test was much higher than recommended, and ranged from 6280 to 7980 lux; OPPTS guidelines suggest that the intensity for *Selenastrum capricornutum* be maintained at 4300 lux.

The pH in the definitive test was much higher than recommended by OPPTS guidelines, and ranged from 7.5-9.0; OPPTS guidelines suggest a pH of 7.5 ± 0.1 for tests with *Selenastrum capricornutum*. The study authors did measure pH in test solutions without algae present, and obtained a range of 7.5 to 8.4.

The 72-hour toxicity values were also calculated by the study authors.

The in-life phase of the definitive algal toxicity test was conducted from September 17 to 21, 2001.

F. CONCLUSIONS:

This study is scientifically sound and is classified as Supplemental. Reported results can be used qualitatively in risk characterization but cannot be used quantitatively for risk estimation.

Biomass was the most sensitive endpoint, with NOAEC and EC_{50} values of <0.065 and 4.6 mg ai/L, respectively. The reviewer's analysis detected a significant effect ($p<0.05$) of the solvent on algal cell density and biomass parameters and noted that the fit of the Bruce-Versteeg model to the data for cell density and biomass was poor and not representative of the raw data.

Test Organism: *Selenastrum capricornutum*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density

IC_{05}:	NA*	95% C.I.:	NA
IC_{50}:	5.4 mg ai/L	95% C.I.:	4.8 to 6.1 mg ai/L

Slope: 3.09 ± 0.653
NOAEC: 0.156 mg ai/L
*NA – value estimated by the Bruce-Versteeg method was not representative of the data and the reviewer recommends not reporting or using this value

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Biomass (Area Under the Growth Curve)
IC_{05}: NA* 95% C.I.: NA
IC_{50}: 4.6 mg ai/L 95% C.I.: 4.1 to 5.1 mg ai/L
Slope: 3.39 ± 0.624
NOAEC: <0.065 mg ai/L
*NA – value estimated by the Bruce-Versteeg method was not representative of the data and the reviewer recommends not reporting or using this value

Growth Rate
IC_{05}: 4.5 mg ai/L 95% C.I.: 3.8 to 5.3 mg ai/L
IC_{50}: >5.56 mg ai/L 95% C.I.: N/A
Slope: 5.69 ± 2.32
NOAEC: 0.156 mg ai/L

Endpoint(s) Effected: Cell density, biomass, and growth rate

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
-------------------------------------------------------------------------------
 GRP1 (SOLVENT CRTL) MEAN =   361.0000     CALCULATED t VALUE =     2.9260
 GRP2 (BLANK CRTL) MEAN   =   342.7667     DEGREES OF FREEDOM =     4
 DIFFERENCE IN MEANS      =    18.2333
-------------------------------------------------------------------------------
TABLE t VALUE (0.05 (2), 4) =  2.776**   SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2), 4) =  4.604    NO significant difference at alpha=0.01


TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
-------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.809          6.534            10.314          6.534       1.809
OBSERVED     0              9                 6             12           0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =  10.9257
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------------------

D = 8060.547

W =    0.948

Critical W (P = 0.05) (n = 27) = 0.923
Critical W (P = 0.01) (n = 27) = 0.894
-------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c        Transform: NO TRANSFORMATION
```


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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 534.56
Closest, conservative, Table H statistic = 2432.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 2
Actual values ==> R (# groups) = 9, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 14.71
Table Chi-square value = 20.09 (alpha = 0.01)
Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 8

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	122012.393	15251.549	34.058
Within (Error)	18	8060.547	447.808	
Total	26	130072.940		

Critical F value = 2.51 (0.05,8,18)
Since F > Critical F REJECT Ho:All groups equal

TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	361.000	361.000		
2	0.065	351.267	351.267	0.563	
3	0.156	352.533	352.533	0.490	
4	0.300	300.567	300.567	3.498	*
5	0.613	333.067	333.067	1.617	
6	1.17	306.400	306.400	3.160	*
7	2.40	294.367	294.367	3.856	*
8	4.79	200.867	200.867	9.268	*
9	5.56	155.833	155.833	11.874	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.065	3	44.578	12.3	9.733
3	0.156	3	44.578	12.3	8.467
4	0.300	3	44.578	12.3	60.433
5	0.613	3	44.578	12.3	27.933
6	1.17	3	44.578	12.3	54.600
7	2.40	3	44.578	12.3	66.633
8	4.79	3	44.578	12.3	160.133
9	5.56	3	44.578	12.3	205.167

TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	361.000	361.000	361.000
2	0.065	3	351.267	351.267	351.900
3	0.156	3	352.533	352.533	351.900
4	0.300	3	300.567	300.567	316.817
5	0.613	3	333.067	333.067	316.817
6	1.17	3	306.400	306.400	306.400
7	2.40	3	294.367	294.367	294.367
8	4.79	3	200.867	200.867	200.867
9	5.56	3	155.833	155.833	155.833

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TR-6 & S. capricornutum 96-hr cell density; mg ai/L
File: 7006c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	361.000				
0.065	351.900	0.527		1.73	k= 1, v=18
0.156	351.900	0.527		1.82	k= 2, v=18
0.300	316.817	2.557	*	1.85	k= 3, v=18
0.613	316.817	2.557	*	1.86	k= 4, v=18
1.17	306.400	3.160	*	1.87	k= 5, v=18
2.40	294.367	3.856	*	1.87	k= 6, v=18
4.79	200.867	9.268	*	1.88	k= 7, v=18
5.56	155.833	11.874	*	1.88	k= 8, v=18

s = 21.161
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.6	0.95	2.7	0.11	0.60
EC10	2.1	1.4	3.1	0.085	0.67
EC25	3.3	2.6	4.1	0.046	0.80
EC50	5.4	4.8	6.1	0.024	0.89

Slope = 3.09 Std.Err. = 0.653

Goodness of fit: p = 0.062 based on DF= 6.0 18.

7006C : TR-6 & S. capricornutum 96-hr cell density; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	361.	336.	24.7	100.	0.00
0.0650	3.00	351.	336.	15.0	100.	1.47e-07
0.156	3.00	353.	336.	16.2	100.	9.62e-05
0.300	3.00	301.	336.	-35.7	100.	0.00513
0.613	3.00	333.	336.	-2.65	99.8	0.171
1.17	3.00	306.	330.	-23.3	98.0	1.97
2.40	3.00	294.	290.	3.96	86.4	13.6
4.79	3.00	201.	191.	10.0	56.7	43.3
5.56	3.00	156.	164.	-8.30	48.8	51.2

TR-6 & S. capricornutum 96-hour biomass; mg ai/L

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File: 7006b Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1017.3333	CALCULATED t VALUE =	3.8275
GRP2 (BLANK CRTL) MEAN =	863.3333	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	154.0000		

TABLE t VALUE (0.05 (2), 4) = 2.776** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.809	6.534	10.314	6.534	1.809
OBSERVED	0	9	8	10	0

Calculated Chi-Square goodness of fit test statistic = 6.9064
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 52173.333

W = 0.953

Critical W (P = 0.05) (n = 27) = 0.923
Critical W (P = 0.01) (n = 27) = 0.894

Data PASS normality test at P=0.01 level. Continue analysis.

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 21.16
Closest, conservative, Table H statistic = 2432.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 2

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```
Actual values    ==>     R (# groups) =   9,    df (# avg reps-1) =   2.00

------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
------------------------------------------------------------------------------

Calculated B statistic =    5.54
Table Chi-square value =   20.09  (alpha = 0.01)
Table Chi-square value =   15.51  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   8
------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b        Transform: NO TRANSFORMATION

                              ANOVA TABLE
------------------------------------------------------------------------------

SOURCE             DF              SS                MS             F
------------------------------------------------------------------------------
Between             8         1173791.333       146723.917        50.620

Within (Error)     18           52173.333         2898.519
------------------------------------------------------------------------------
Total              26         1225964.667
------------------------------------------------------------------------------

  Critical F value =   2.51  (0.05,8,18)
  Since  F > Critical F  REJECT  Ho:All groups equal


TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
-----  --------------------    ----------    ------------------  ------  ---
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1	Neg control	1017.333	1017.333		
2	0.065	844.000	844.000	3.943	*
3	0.156	817.000	817.000	4.557	*
4	0.300	794.333	794.333	5.073	*
5	0.613	859.000	859.000	3.602	*
6	1.17	808.333	808.333	4.754	*
7	2.40	716.333	716.333	6.847	*
8	4.79	427.667	427.667	13.414	*
9	5.56	318.000	318.000	15.909	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.065	3	113.413	11.1	173.333
3	0.156	3	113.413	11.1	200.333
4	0.300	3	113.413	11.1	223.000
5	0.613	3	113.413	11.1	158.333
6	1.17	3	113.413	11.1	209.000
7	2.40	3	113.413	11.1	301.000
8	4.79	3	113.413	11.1	589.667
9	5.56	3	113.413	11.1	699.333

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	1017.333	1017.333	1017.333
2	0.065	3	844.000	844.000	844.000
3	0.156	3	817.000	817.000	823.444
4	0.300	3	794.333	794.333	823.444
5	0.613	3	859.000	859.000	823.444
6	1.17	3	808.333	808.333	808.333
7	2.40	3	716.333	716.333	716.333
8	4.79	3	427.667	427.667	427.667
9	5.56	3	318.000	318.000	318.000

TR-6 & S. capricornutum 96-hour biomass; mg ai/L
File: 7006b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

US EPA ARCHIVE DOCUMENT

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	1017.333				
0.065	844.000	3.943	*	1.73	k= 1, v=18
0.156	823.444	4.411	*	1.82	k= 2, v=18
0.300	823.444	4.411	*	1.85	k= 3, v=18
0.613	823.444	4.411	*	1.86	k= 4, v=18
1.17	808.333	4.754	*	1.87	k= 5, v=18
2.40	716.333	6.847	*	1.87	k= 6, v=18
4.79	427.667	13.414	*	1.88	k= 7, v=18
5.56	318.000	15.909	*	1.88	k= 8, v=18

s = 53.838
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.5	0.93	2.4	0.099	0.62
EC10	1.9	1.3	2.8	0.080	0.68
EC25	2.9	2.3	3.6	0.049	0.79
EC50	4.6	4.1	5.1	0.022	0.90

Slope = 3.39 Std.Err. = 0.624

!!!Poor fit: p = 0.0095 based on DF= 6.0 18.

7006B : TR-6 & S. capricornutum 96-hour biomass; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	1.02e+03	861.	156.	100.	0.00
0.0650	3.00	844.	861.	-17.2	100.	1.99e-08
0.156	3.00	817.	861.	-44.2	100.	3.39e-05
0.300	3.00	794.	861.	-66.9	100.	0.00308
0.613	3.00	859.	860.	-0.893	99.8	0.156
1.17	3.00	808.	842.	-33.6	97.8	2.25
2.40	3.00	716.	714.	2.58	82.9	17.1
4.79	3.00	428.	408.	20.0	47.3	52.7
5.56	3.00	318.	334.	-15.7	38.7	61.3

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	65.3333	CALCULATED t VALUE =	2.4749
GRP2 (BLANK CRTL) MEAN =	60.6667	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	4.6667		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

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TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.809	6.534	10.314	6.534	1.809
OBSERVED	0	11	5	11	0

Calculated Chi-Square goodness of fit test statistic = 12.4609
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 50.667

W = 0.977

Critical W (P = 0.05) (n = 27) = 0.923
Critical W (P = 0.01) (n = 27) = 0.894

Data PASS normality test at P=0.01 level. Continue analysis.

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 19.00
Closest, conservative, Table H statistic = 2432.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 2
Actual values ==> R (# groups) = 9, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

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TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 6.76
Table Chi-square value = 20.09 (alpha = 0.01)
Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 8

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	262.519	32.815	11.657
Within (Error)	18	50.667	2.815	
Total	26	313.185		

Critical F value = 2.51 (0.05,8,18)
Since F > Critical F REJECT Ho:All groups equal

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	65.333	65.333		
2	0.065	64.667	64.667	0.487	
3	0.156	63.333	63.333	1.460	
4	0.300	61.333	61.333	2.920	*
5	0.613	62.000	62.000	2.433	
6	1.17	62.000	62.000	2.433	
7	2.40	63.667	63.667	1.217	
8	4.79	58.667	58.667	4.866	*
9	5.56	54.667	54.667	7.786	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

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TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.065	3	3.534	5.4	0.667
3	0.156	3	3.534	5.4	2.000
4	0.300	3	3.534	5.4	4.000
5	0.613	3	3.534	5.4	3.333
6	1.17	3	3.534	5.4	3.333
7	2.40	3	3.534	5.4	1.667
8	4.79	3	3.534	5.4	6.667
9	5.56	3	3.534	5.4	10.667

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	65.333	65.333	65.333
2	0.065	3	64.667	64.667	64.667
3	0.156	3	63.333	63.333	63.333
4	0.300	3	61.333	61.333	62.250
5	0.613	3	62.000	62.000	62.250
6	1.17	3	62.000	62.000	62.250
7	2.40	3	63.667	63.667	62.250
8	4.79	3	58.667	58.667	58.667
9	5.56	3	54.667	54.667	54.667

TR-6 & S. capricornutum 96-hr growth rate; mg ai/L
File: 7006g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	65.333				
0.065	64.667	0.487		1.73	k= 1, v=18
0.156	63.333	1.460		1.82	k= 2, v=18
0.300	62.250	2.251	*	1.85	k= 3, v=18
0.613	62.250	2.251	*	1.86	k= 4, v=18
1.17	62.250	2.251	*	1.87	k= 5, v=18
2.40	62.250	2.251	*	1.87	k= 6, v=18
4.79	58.667	4.867	*	1.88	k= 7, v=18
5.56	54.667	7.787	*	1.88	k= 8, v=18

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s = 1.678
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	4.5	3.8	5.3	0.035	0.85
EC10	5.2	4.8	5.5	0.015	0.93
EC25	6.6	5.5	8.0	0.039	0.83
EC50	8.7	5.8	13.	0.086	0.66

Slope = 5.69 Std.Err. = 2.32

Goodness of fit: p = 0.082 based on DF= 6.0 18.

7006G : TR-6 & S. capricornutum 96-hr growth rate; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	65.3	63.2	2.14	100.	0.00
0.0650	3.00	64.7	63.2	1.47	100.	2.25e-14
0.156	3.00	63.3	63.2	0.140	100.	2.25e-14
0.300	3.00	61.3	63.2	-1.86	100.	2.25e-14
0.613	3.00	62.0	63.2	-1.19	100.	2.94e-09
1.17	3.00	62.0	63.2	-1.19	100.	3.70e-05
2.40	3.00	63.7	63.1	0.520	99.9	0.0746
4.79	3.00	58.7	58.7	-0.0513	92.9	7.08
5.56	3.00	54.7	54.6	0.0245	86.5	13.5

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

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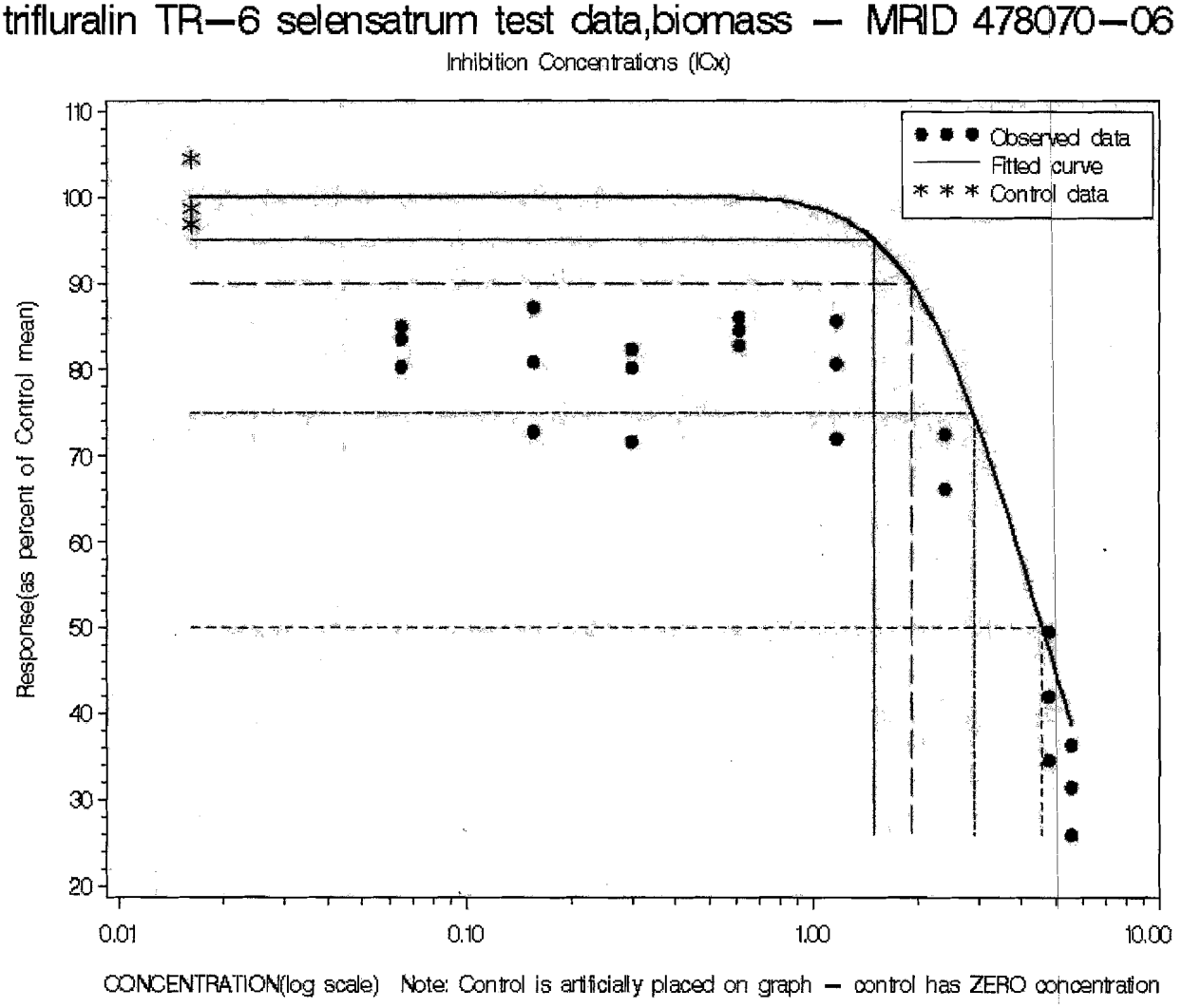

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